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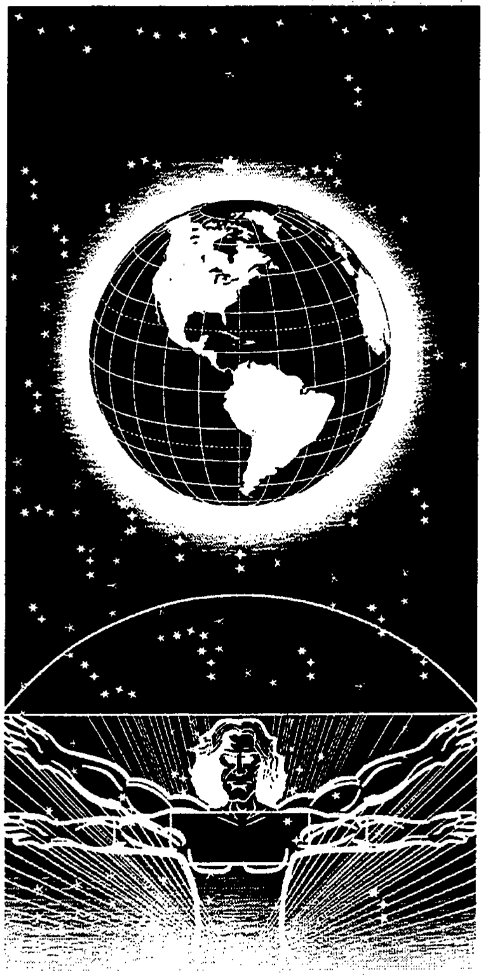

UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

GENERAL TOXICITY/REPRODUCTIVE TOXICITY SCREEN OF MODULAR ARTILLERY CHARGE SYSTEM (XM232) ADMINISTERED IN THE DIET OF SPRAGUE-DAWLEY RATS

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TECHNICAL REVIEW AND APPROVAL

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

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An artillery propellant under development by the US Army is a granular mixture of 98% nitrocellulose, nitroglycerine, nitroguanidine, and <2% cryolite and graphite. The propellant, Modular Artillery Charge System, or MACS, consists of a single increment of propellant charge contained within a rigid, combustible casing. As part of the process to develop environmental and health effects criteria, a 90-day modified Screening Information Data Set reproductive assay was performed. Male and Female Sprague-Dawley rats were treated with diet containing either 0.0, 0.2, 1.0 or 2.0 g propellant/kg diet. No mortalities occurred and body weights were unaffected by treatment. Methemoglobin concentrations of high-dose rats measured after 28 days and at the conclusion of the study were significantly elevated (23-25%) compared to control rats. Relative organ weights of treated animals did not differ from weights of their respective control groups. No adverse effects occurred in mating or fertility indices. No significant treatment related differences were noted in length of gestation, sex ratio, gestation index, or mean number of pups per litter.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc., located at Wright-Patterson Air Force Base, OH. This document serves as a final report on the reproductive toxicity screen of Modular Artillery Charge System administered in the diet of Sprague-Dawley Rats. The research described in this report began in September 1994 and was completed in October 1995 under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. A08). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division. This study was sponsored by the U.S. Army under the direction of LTC Daniel J. Caldwell, USAMRD/WRAIR.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

µL	Microliter
ALT	Alanine aminotransaminase
AST	Aspartate aminotransaminase
BUN	Blood urea nitrogen
dL	Deciliter
fL	Femtoliter
g	Gram
G	Gestation
GGT	Gamma-Glutamyl transferase
GTN	Nitroglycerin
h	Hour
HCT	Hematocrit
HGB	Hemoglobin
IP	Intraperitoneal
IU	International unit
kg	Kilogram
L	Lactation; Liter
MACS	Modular Artillery Charge System
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
MetHb	Methemoglobin
mg	Milligram
min	Minute
mm	Millimeter
mmol	Millimole
N	Number
NC	Nitrocellulose
nm	Nanometer
NQ	Nitroquanidine
p	Probability
PD	Predosing
pg	Picogram

PM	Premating
PW	Postweaning
RBC	Red blood cell
rpm	Revolutions per minute
SEM	Standard error of the mean
UV	Ultraviolet
WBC	White blood cell
wt	Weight

SECTION 1

INTRODUCTION

Modular Artillery Charge System (MACS; XM232) is a propellant under development by the US Army for autoloading howitzer artillery consisting of a single increment of propellant charge contained within a rigid combustible casing. This technology will simplify autoloader requirements, result in a significant reduction in propellant production and logistics requirements, and eliminate propellant waste.

The MACS propellant is a granular mixture of nitrocellulose (NC), nitroglycerin (GTN; glyceryl trinitrate), nitroguanidine (NQ), ethylcentralite (N,N'diethyl, N,N'diphenylurea), and potassium sulfate. Since NC, GTN, and NQ make up approximately 98% of MACS propellant, it is commonly referred to as "triple-based" propellant mixture.

Nitroguanidine is a primary component (approximately 48%) of the triple-based propellant mixture. Previously conducted acute oral and percutaneous studies on NQ found the compound to be relatively nontoxic. The compound was not irritating to either rabbit skin or eyes using the standard Draize technique (Hiatt et al., 1986; Morgan et al., 1986). Nitroguanidine did not kill rats by oral gavage at a limit test concentration of 5 g/kg (Brown et al., 1988), while the oral LD_{50}s for male and female mice are reported as >5.0 g/kg and 4.3 g/kg, respectively (Hiatt et al., 1988).

Nitroguanidine did not produce treatment-related effects when administered in the diet of Sprague-Dawley rats at doses as high as 1000 mg/kg/day for 14 days. However, after 90 days that dose level produced a decrease in body weight gain and food consumption (Reddy and Korte, 1988). No evidence of developmental toxicity in rats or rabbits was reported following conditions of the above study.

Nitroglycerin is also a primary component (approximately 22%) of the triple-based propellant. The toxic, physiologic, and pharmacologic effects of GTN have been studied extensively in animals and man (NIOSH, 1978). Nitroglycerin is readily absorbed by ingestion, inhalation, sublingual contact, and through the skin. When both inhalation and dermal exposure to GTN occur, dermal contact is thought to make the major contribution to the total amount absorbed by the body (NIOSH, 1978). Vasodilatation following exposure to GTN can occur within minutes, regardless of the route of exposure. This effect has led to sudden death and chronic cardiac disease in humans (Carmichael and Lieben, 1963). Small amounts can cause headaches which can persist for hours or days. Larger doses can cause hypotension, cyanosis, and methemoglobinemia. These effects are potentiated by alcohol consumption (NIOSH, 1978). Symptoms also include nausea and vomiting, and in some instances, diarrhea. The mouse intraperitoneal (IP) LD_{50} of GTN is 194 mg/kg (Kylin et al., 1964) and the rat IP LD_{50} has been reported as 93 mg/kg (Burginson et al., 1962).

The third major component (approximately 28%) of the triple-based propellant is nitrocellulose or cellulose nitrate. Cellulose nitrate is relatively nontoxic but is known for its extreme flammability (Montgomery, 1982). Dry NC may explode when subjected to heat or sudden shock; NC is therefore generally handled wet with either water or alcohol. Toxic combustion products (primarily carbon monoxide and oxides of nitrogen) are generated when the compound is ignited (Montgomery, 1982).

Munition workers exposed to nitrate-containing explosives have experienced skin irritation, liver damage, and anemia (Hathaway, 1977; Morton et al., 1976; Stewart et al., 1945). These nitrate-containing compounds have also been found to cause methemoglobin formation, liver and spleen hypertrophy, and degeneration of the seminiferous tubules resulting in decreased spermatogenesis (Cody et al., 1981; Levine et al., 1983, 1984; Furedi et al., 1984a,b). A recently completed reproductive screen in this laboratory with a nitrate-containing explosive compound resulted in testicular atrophy and decreased spermatogenesis in the male rats (Kinkead et al., 1994, 1995a). Female rats displayed clinical signs of altered locomotion during the postpartum period and both sexes had brain lesions at necropsy. Neurotransmitter analysis showed statistically significant increases in norepinephrine, epinephrine, seratonin, and dopamine in the 1,3,5-trinitrobenzene (TNB) treated rats (Narayanan et al., 1995). Changes in neurotransmitter levels in specific regions may be one of the mechanisms responsible for TNB induced neurological disorder. A reproductive

screen on other nitrate containing explosive compounds resulted in hemolytic anemia for Liquid Propellant XM46 (Kinkead et al., 1995b), and loss of embryos and/or death of fetuses postmating for ammonium dinitramide (Kinkead et al., 1995c).

Nitrate-containing explosive compounds have produced adverse reproductive effects in both male and female rats. Testicular atrophy and decreased spermatogenesis are common findings in male rats; lack of live litter production has been noted in female rats. Brain lesions and loss of motor skills, methemoglobinemia, and hemolytic anemia have been common findings in the reproductive screens performed within this laboratory. Because of the above findings, a 90-day reproductive screen was performed on MACS to evaluate its potential to produce alterations in paternal fertility, maternal pregnancy and lactation, and growth and development of offspring.

A dose range-finding study was first performed in which MACS was administered in the diet of male and female Sprague-Dawley rats for a three-week period. The rats received diet containing either 0.0, 0.25, 0.5, 1.0, or 2.0 g propellant/kg diet (Kinkead et al., 1995d). The high concentration was set at 2.0 g/kg with the consideration that MACS consist of nearly 50% NQ, and NQ at doses of 1.0 g/kg produced decreased body weight gains and food consumption after 90-days treatment (Reddy and Korte, 1988). No mortalities occurred during the study and body weights were unaffected by the treatment. Methemoglobin concentrations measured at the conclusion of the treatment period indicated no

differences between treated and control animals. Relative liver weights of the high-dose female rats were significantly ($p<0.01$) greater than the control group.

Based on the results of the dose range-finding study, the doses selected for the 90-day reproductive screen were 0.0, 0.2, 1.0, and 2.0 g MACS/kg body weight.

SECTION 2

MATERIALS AND METHODS

Test Agent

The MACS was provided by the U.S. Army, Picatinny Arsenal, NJ. The pellets were ground to granular form prior to receipt. Pertinent chemical and physical properties of the test compound are listed below:

MACS

Synonyms:	Smokeless powder Modular Artillery Charge System UNICHARGE/XM232/M30/M30A1
CAS No.	None assigned
Specific gravity:	1.5880
Vapor pressure:	Negligible
Appearance:	Hard cylindric pellet, white to reddish orange, coated with graphite

Diet Preparation, Homogeneity, Stability, and Analysis

MACS was administered by the oral route, mixed appropriately in the diet. The test material was mixed in powdered Purina Formulab #5002 (Ralston Purina, St. Louis, MO) certified rodent diet meal. The diet was prepared by adding the appropriate amount of MACS to 10 kg rodent diet and mixing using a Hobart mixer (Model No. H600T, Hobart Corp., Dayton, OH) for a minimum of 90 minutes. MACS analysis was performed using a high performance liquid chromatographic method described in Appendix A. Total MACS concentrations were based on NQ concentrations measured in 5g samples of diet.

To assess the efficiency of the mixing process, analyses were performed on samples taken from the top, middle, and bottom of the mixing bowl. Duplicate samples were taken for each measurement. Stability analyses were performed on diet stored under ambient conditions in polyethylene containers for up to 21 days (Kinkead et al., 1995d).

Group Assignments and Dose Levels

Group	Number of Animals: Male	Number of Animals: Female	Target Dose Levels of MACS (mg/kg diet)[a]	Target Dose of MACS (mg/kg body weight/day)[b]
Control	12	12	0.0	0.0
Low	12	12	200.0	12.0
Middle	12	12	1000.0	60.0
High	12	12	2000.0	120.0

[a]Target dose levels based on range-finding results. The highest dose level produced relative liver weight increases in female rats (Kinkead et al., 1995d).

[b]Assumes that a 500 g rat consumes 30 g feed/day.

Test Animals and Clinical Measurements

Fifty male and 50 female Sprague-Dawley derived outbred albino rats [Crl:CD[R](sd)BR], known as Charles River CD rats, were purchased from Charles River Breeding Laboratories, Raleigh, NC. The rats were 8 weeks of age upon arrival and 11 weeks of age at initiation of the treatment period. All rats were identified by tail tattoo and were acclimatized for two weeks. During the acclimation period, quality control procedures were performed on selected rats as described in

Kinkead et al. (1991) Rodent water and feed were available *ad libitum.* Animal room temperatures were targeted at 21 to 25 °C, and the light/dark cycle was set at 12-h intervals. Parental rats were single housed (except for the mating period) in clear plastic cages with hardwood-chip bedding (Bettachip[R], Northeastern Products Corp., Warrensburg, NY). During the mating period the animals were housed in clear plastic cages with stainless steel wire bottoms. There were 4 study groups with target doses of 0, 200, 1000, and 2000 mg MACS/kg diet. Rats were assigned to groups consisting of 12 females and 12 males by means of a computer-generated randomization. The randomization was stratified by body weight such that the mean body weights of all groups were homogeneous by statistical analysis at study initiation.

Rats were observed twice daily for signs of toxic stress. Male rat body weights were measured weekly during the 90-day study. Female body weights were measured in the same manner until confirmation of mating. During gestation, females were weighed on Gestation Days 0, 4, 7, 14, and 20. Dams producing litters were weighed on Postpartum Days 0, 4, 7, 14, and 21, then weekly thereafter.

Food consumption was determined during the prebreeding period for both male and female rats. Food consumption of individual dams was measured for Gestation Days 0 to 7, 7 to 14 and 14 to 20, and for Postpartum Days 0 to 7 and 7 to 14. Male food consumption was calculated weekly through 90 days. Food consumption was not measured during the mating period when more than one rat was in a cage or during

Postpartum Days 14 to 21 when pups were beginning to eat from the feed jars. Feed jars were cleaned on a weekly basis at which time all leftover food was discarded. Food consumption was measured every two/three days until the postpartum period when it was measured daily in the female rats. The live and dead pups were counted and sexed on Postpartum/Lactation Day 0. All pups were counted and sexed, and live pups were weighed 1, 4, 7, 14, and 21 days after birth. Standardization of litter sizes, 4 per sex when possible, occurred on Day 4. Pups were examined for external abnormalities.

General Study Design

Six male rats per group were dosed from 14 days prior to mating and throughout the mating period for a total of 28 days. The remaining 6 male rats per group were treated in the same manner for 90 days. All female rats were dosed from 14 days prior to mating, during mating and gestation, postpartum (21 days), and for 4 weeks postweaning for a total of 90 days. Pups were maintained on MACS-treated diet through 4-weeks postweaning.

One male and one female were cohabited, selected randomly from within their respective dose groups, starting on study Day 14. The pairs remained cohabited for a maximum of 14 days, or until either a copulation plug was present or sperm were found in the vaginal wash. The day a copulation plug was present or sperm were found in the vaginal wash was defined as Gestation Day 0.

At necropsy, blood samples were taken via the vena cava from parental animals for hematology and clinical chemistry assays listed in Table 1. Erythrocytes were enumerated on a Coulter counter (Coulter Electronics, Hialeah, FL) and sera for clinical chemistry evaluations were assayed on an Ektachem 250 (Eastman Kodak, Rochester, NY). Selected hematological parameters and absolute leukocyte differentials were determined according to established procedures. Sera were processed according to the procedures in the Ektachem Operations manual. Methemoglobin assays were performed using a Model IL282 cooximeter (Instrumentation Laboratory, Lexington, MA).

Opto-Varimex open-field activity evaluation tests (Columbus Instruments International Corporation, Columbus, OH) were performed on the parental rats. Tests were performed on male rats prior to dosing, postmating, and prior to sacrifice; on dams prior to dosing, during the postpartum period, and again prior to sacrifice. Four test chambers were used in the Opto-Varimex test, each having an observation area of approximately 17 x 17.5 inches, with sensors at 1-inch intervals (15 per side). Test sessions consisted of 5 consecutive 2-min intervals. Measurements included total distance traveled, time spent resting, time ambulatory, and time spent in "stereotypic movement" which included rearing and circling. The number of clockwise and counterclockwise rotations was tabulated. Following each session, the chambers were sprayed with a disinfectant/deodorant and wiped clean.

Evaluations at Necropsy

Brain, liver, kidneys, spleen, thymus, testes, and epididymides were weighed at necropsy. Sperm count and motility were also evaluated. Sperm were removed from the right cauda epididymis and analyzed microscopically using a videomicrography system (Cell Soft Automated Semen Analyzer, Cryo Resources, Ltd., Montgomery, NY) (Toth et al., 1992). Bouin's fixative was used to fix the testes and epididymides. The pituitary, spleen, liver, kidneys, bone marrow, and reproductive organs were removed from parental animals of both sexes and fixed in 10% buffered formalin solution. After routine processing, the tissues were embedded in paraffin and stained with hematoxylin and eosin for histopathologic examination. Pups were examined for gross lesions at necropsy.

Statistical Analysis

Pup weights, organ weights, organ weight ratios, serum chemistry, hematology, and MACS dose calculations were analyzed for statistical significance using a one-factorial analysis of variance with Bonferroni multiple comparisons (Rosner, 1990). A one-factorial repeated measures analysis of variance with Bonferroni multiple comparisons was used for food consumption, paternal and maternal body weights (Barcikowski, 1983). Mating indices and histopathologic results were analyzed by a Chi-square test of proportions applied to the incidence data (Rosner,

1990). Tissue lesion severity data were analyzed using the Kruskal-Wallis analysis of variance (Rosner, 1990).

Sperm analysis and Opto-Varimex data were analyzed using one-way analysis of variance, employing Dunnett's technique (Sokal and Rohlf, 1981) for multiple comparisons between control and treatment animals when significant differences ($p<0.05$) occurred. Parametric analysis techniques were performed when possible. However, when transformation techniques failed to present a normal distribution, a Kruskal-Wallis rank-based analysis of variance (Sokal and Rohlf, 1981) was used.

SECTION 3

RESULTS

Food Consumption and Calculated Dose

Food consumption of the male rats remained relatively constant throughout the 90-day study (Table 2). Food consumption of the female rats increased during the postpartum (lactation) period compared to the premating and gestation periods (Table 3). Male rats consumed approximately 35 g diet/day which resulted in a mean dose of 127, 72, and 14 mg MACS/kg/day in the high-, mid-, and low-dose groups, respectively. Female rats received a median dose of 161, 93, and 19 mg MACS/kg/day for the high-, mid-, and low-dose groups, respectively.

General Toxicity

No mortality occurred in the parental animals during the study. Mean body weights of the treated rat groups did not differ from the mean body weights of their respective control groups (Tables 4 and 5). No clinical signs of toxic stress or of motor skill loss were noted during the study. This was confirmed by the Opto-Varimex tests in which no differences in locomotor skills were detected in treated or control rats.

Six male rats per group were necropsied following the mating period (28 days of treatment) and the remaining six per group were necropsied at study termination (90 days of treatment). No treatment-

related differences were noted in absolute or relative organ weights in male rats at either of the evaluation periods (Tables 6 and 7). Likewise, no treatment-related differences were noted in absolute or relative organ weights of female rats examined after 90 days of treatment (Table 8).

Male rats sacrificed following mating (28 days) and after 90 days of treatment showed no adverse effects on sperm function/activity. All measurements of sperm function/activity evaluated in the MACS-treated rats were similar to the sperm function of the control rats (Table 9).

Methemoglobin concentration was significantly ($p<0.01$) increased in the high-dose male rats following 28 and 90 days of treatment (Tables 10 and 11). A similar increase in methemoglobin concentration was noted in the mid- and high-dose female rats examined following 90 days (Table 12). All hematology measurements were within normal value ranges.

After 28 days of treatment, alanine aminotransaminase (ALT) values of the high-dose male rats were significantly ($p<0.05$) lower than control values (Table 13). The ALT value of high-dose male rats was also significantly ($p<0.05$) less than control values following 90 days of treatment (Table 14); however, this difference was not noted in female rats (Table 15).

At necropsy, all animals were judged to be in good general condition. Renal cysts were observed in the left kidneys of one low-

dose and one control female rat. Hydronephrosis was observed in the kidneys of two control male rats. Unilateral testicular atrophy was noted in a control rat and reticulated yellow mottling of the liver was observed in a mid-dose male rat.

Histopathology

Observed histopathologic lesions were considered to be incidental findings unrelated to treatment. Statistical analysis revealed a significant ($p<0.05$) increased incidence of renal hyaline casts in the low-dose female rats. The finding was not dose-related and is not considered to be of biological significance. In all groups, minimal (early) nephropathy changes were a common finding in male rats, as was renal mineralization in female rats.

Reproductive Indices

The treatment produced no adverse effects on mating as 100% of the animals mated (Table 16). The fertility index was 100% in the mid-dose group and 92% in the other treated and control groups. No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of offspring per litter. The mean body weights of male and female pups from the MACS-treated dams did not differ from the mean weights of their respective controls (Table 17).

SECTION 4

DISCUSSION

Administering MACS (XM232) in the diet of Sprague-Dawley rats at calculated dose levels of 127, 72, and 14, or 161, 93 and 19 mg MACS/kg body weight/day to males and females, respectively, produced no adverse effects on reproductive performance or litter parameters. No treatment-related effects in mean pup body weights were noted during the weaning or postweaning time periods.

Anemia and increased methemoglobin production are common characteristics of nitrate poisoning. Hemolytic anemia and methemoglobinemia have been reported for male and female Sprague-Dawley rats treated with the nitrate-containing liquid propellant XM46 at dosages as low as 17 mg XM46/kg/day (Kinkead et al., 1995b). Anemia was not noted in this study but methemoglobin concentrations were increased slightly in the high-dose male rats and the mid- and high-dose female rats. Although the increases in methemoglobin were statistically significant, the effects would be considered to be of little biological significance. In an oral study of guanidine nitrate (Mullen et al., 1988), the only treatment-related gross finding was multiple red foci in the thymus, presumably hemorrhage. Thymic hemorrhage was not a treatment-related finding in this study. There was also no treatment-related effects in body weight gain or food consumption.

SECTION 5

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Table 1. Serum Chemistry and Whole Blood Assessments from Control and MACS-Treated Sprague-Dawley Rats

Serum Chemistry	Whole Blood Assessments
Albumin	Methemoglobin
Alkaline phosphatase	Platelet count
Alanine aminotransaminase	Hematocrit
Aspartate aminotransaminase	Hemoglobin
Bilirubin	Red blood cell count
Blood Urea Nitrogen	Total and differential leukocyte count
Creatinine	
Creatine kinase	
CO_2	
Chloride	
Calcium	
Gamma-Glutamyl transferase	
Globulin	
Glucose	
Potassium	
Phosphorus	
Sodium	
Total protein	
Magnesium	
Triglycerides	

Table 2. **Mean Daily Food Consumption[a] of Male Rats Treated with MACS for 90 Days**

DAY	CONTROL	LOW	MEDIUM	HIGH
2	28 ± 1	29 ± 1	32 ± 3	31 ± 2
4	30 ± 1	30 ± 1	31 ± 1	30 ± 1
6	30 ± 1	29 ± 1	30 ± 1	28 ± 2
8	27 ± 2	28 ± 1	29 ± 1	28 ± 1
10	30 ± 1	30 ± 1	31 ± 1	30 ± 1
12	32 ± 2	31 ± 1	33 ± 2	33 ± 3
14	34 ± 2	32 ± 2	35 ± 2	33 ± 2
22	39 ± 3	33 ± 2	35 ± 2	35 ± 3
23	37 ± 4	35 ± 2	35 ± 2	36 ± 3
24	34 ± 2	33 ± 2	40 ± 4	36 ± 4
25	34 ± 3	32 ± 3	34 ± 2	33 ± 3
26	36 ± 3	34 ± 2	32 ± 2	35 ± 3
27	37 ± 4	31 ± 2	40 ± 4	36 ± 3
28	34 ± 3	37 ± 4	34 ± 2	32 ± 2
29	40 ± 4	37 ± 3	36 ± 3	40 ± 4
30	34 ± 3	28 ± 1	38 ± 3	33 ± 1
31	37 ± 3	32 ± 2	35 ± 2	33 ± 2
32	34 ± 3	33 ± 5	37 ± 3	32 ± 1
33	32 ± 6	33 ± 2	43 ± 7	37 ± 1
34	36 ± 3	31 ± 4	36 ± 2	34 ± 2
35	37 ± 4	31 ± 4	35 ± 2	37 ± 3
36	35 ± 4	32 ± 5	34 ± 3	30 ± 2
37	38 ± 5	28 ± 3	34 ± 3	32 ± 4
38	40 ± 6	34 ± 6	34 ± 2	33 ± 3
39	32 ± 3	25 ± 2	39 ± 3	30 ± 2
40	32 ± 4	27 ± 5	40 ± 5	35 ± 6
41	35 ± 6	26 ± 2	36 ± 3	34 ± 4
42	34 ± 3	29 ± 2	36 ± 3	34 ± 2
43	39 ± 4	30 ± 3	42 ± 5	34 ± 3
44	40 ± 4	32 ± 4	38 ± 3	35 ± 3
45	36 ± 3	30 ± 1	36 ± 2	36 ± 5
46	40 ± 7	33 ± 5	41 ± 5	38 ± 4
47	40 ± 4	30 ± 1	35 ± 5	37 ± 4
49	33 ± 1	28 ± 1	36 ± 2	28 ± 2
51	33 ± 2	29 ± 2	34 ± 2	35 ± 1
52	37 ± 5	31 ± 2	37 ± 3	39 ± 6
53	40 ± 6	35 ± 8	42 ± 5	36 ± 4
54	39 ± 4	28 ± 3	38 ± 4	36 ± 4
55	42 ± 5	31 ± 2	38 ± 3	42 ± 4
56	33 ± 4	28 ± 1	35 ± 4	34 ± 4

Table 2. cont'd

DAY	CONTROL	LOW	MEDIUM	HIGH
57	40 ± 9	32 ± 3	34 ± 5	36 ± 9
58	36 ± 4	31 ± 1	35 ± 2	39 ± 9
59	41 ± 5	33 ± 3	33 ± 2	32 ± 7
60	39 ± 7	33 ± 5	44 ± 3	33 ± 7
61	37 ± 5	32 ± 3	36 ± 5	28 ± 6
62	48 ± 8	36 ± 6	38 ± 2	35 ± 8
63	49 ± 9	40 ± 7	36 ± 4	35 ± 7
64	45 ± 7	33 ± 4	36 ± 3	34 ± 7
66	37 ± 3	34 ± 4	33 ± 3	29 ± 6
68	38 ± 3	35 ± 3	34 ± 2	32 ± 5
70	35 ± 1	31 ± 1	31 ± 1	26 ± 5
72	29 ± 2	27 ± 1	28 ± 2	27 ± 2
74	31 ± 1	33 ± 4	29 ± 2	30 ± 1
76	29 ± 1	27 ± 1	28 ± 2	32 ± 2
78	31 ± 1	27 ± 3	32 ± 3	33 ± 2
80	33 ± 1	30 ± 1	32 ± 3	33 ± 2
84	32 ± 2	30 ± 1	30 ± 1	32 ± 2
86	27 ± 1	27 ± 2	29 ± 3	29 ± 1
88	29 ± 1	32 ± 5	31 ± 3	30 ± 1
90	32 ± 1	29 ± 1	33 ± 3	28 ± 1
91	30 ± 3	25 ± 2	30 ± 3	32 ± 0

[a]Mean ± SEM

Table 3. **Mean Food Consumption[a] of Female Rats Treated with MACS for 90 Days**

DAY	CONTROL	LOW	MEDIUM	HIGH
PM2	22 ± 1	23 ± 1	21 ± 1	21 ± 1
PM4	21 ± 1	21 ± 1	21 ± 1	20 ± 1
PM6	25 ± 1	23 ± 1	21 ± 1	21 ± 1
PM8	19 ± 1	18 ± 1	19 ± 1	17 ± 1
PM10	24 ± 1	22 ± 1	22 ± 2	21 ± 1
PM12	21 ± 1	20 ±<1	20 ± 1	19 ± 1
PM14	22 ± 1	23 ± 1	23 ± 1	21 ± 1
G0-7	28 ± 2	26 ± 1	29 ± 1	27 ± 1
G7-14	31 ± 3	28 ± 1	32 ± 2	29 ± 1
G14-20	34 ± 3	32 ± 1	39 ± 3	35 ± 2
L0-7	42 ± 2	42 ± 1	43 ± 2	40 ± 1
L7-14	60 ± 2	64 ± 1	66 ± 2	62 ± 2
PW1	31 ± 3	27 ± 2	30 ± 3	28 ± 3
PW2	27 ± 2	25 ± 1	26 ± 1	26 ± 2
PW3	29 ± 2	26 ± 1	27 ± 1	30 ± 2
PW4	30 ± 2	25 ± 1	27 ± 2	24 ± 2
PW5	26 ± 1	22 ± 1	25 ± 1	24 ± 1
PW6	26 ± 2	22 ± 1	24 ± 2	23 ± 1
PW7	21 ± 1	22 ± 1	22 ± 1	21 ± 1
PW8	20 ± 1	21 ± 1	22 ± 1	22 ± 1
PW9	23 ± 1	23 ± 1	22 ± 1	22 ± 1
PW10	20 ± 1	20 ± 1	21 ± 1	22 ± 1
PW11	19 ± 1	19 ± 1	21 ± 1	21 ± 1
PW12	21 ± 1	21 ± 1	21 ± 1	20 ±<1
PW13	21 ± 1	21 ± 1	21 ± 1	22 ± 1
PW14	19 ± 1	24 ± 1	22 ± 1	22 ± 1

[a]Mean ± SEM, N = 12.
PM = Premating
G = Gestation
L = Lactation
PW = Postweaning

Table 4. Mean Body Weights[a] of Male Rats Treated with MACS for 90 Days

DAY	CONTROL	LOW	MEDIUM	HIGH
-7	369 ± 5.2	369 ± 5.5	362 ± 5.1	366 ± 5.4
0	403 ± 7.1	403 ± 7.2	400 ± 6.6	401 ± 6.6
7	425 ± 8.2	429 ± 8.2	426 ± 8.1	424 ± 8.2
14	446 ± 9.1	452 ± 9.0	449 ± 9.8	443 ± 9.1
21	462 ± 11.0	470 ± 8.6	462 ± 9.4	454 ± 10.1
28	482 ± 11.4	491 ± 10.3	485 ± 11.9	474 ± 10.4
35	502 ± 15.8	494 ± 16.0	510 ± 20.1	509 ± 17.6
42	503 ± 14.9	488 ± 18.0	520 ± 25.0	516 ± 22.3
49	524 ± 15.2	509 ± 17.2	546 ± 25.7	530 ± 22.7
56	536 ± 16.1	518 ± 18.6	559 ± 26.3	552 ± 21.6
63	551 ± 15.5	536 ± 18.7	569 ± 25.6	548 ± 35.5
70	562 ± 16.4	549 ± 15.6	581 ± 26.8	544 ± 46.8
77	565 ± 17.9	551 ± 15.4	585 ± 26.9	571 ± 31.9
84	577 ± 19.5	564 ± 16.1	603 ± 28.0	589 ± 26.1

[a]Mean ± SEM; N = 12 on Days -7 through 28, N = 6 on Days 35 through 84.

Table 5. Mean Body Weights[a] of Female Rats Treated with MACS for 90 Days

DAY	CONTROL	LOW	MEDIUM	HIGH
PD7	255 ± 4.6	257 ± 4.0	256 ± 4.6	253 ± 4.5
PM0	265 ± 5.0	265 ± 3.9	267 ± 6.2	264 ± 4.8
PM7	272 ± 7.2	274 ± 6.0	276 ± 7.1	266 ± 5.5
PM14	276 ± 7.1	278 ± 5.8	273 ± 8.7	269 ± 6.2
G0	276 ± 7.4	277 ± 5.9	286 ± 11.6	276 ± 3.6
G7	318 ± 8.9	322 ± 7.4	341 ± 16.8	321 ± 5.2
G14	353 ± 10.8	355 ± 8.9	351 ± 10.4	350 ± 3.9
G20	434 ± 13.1	439 ± 11.1	441 ± 15.1	421 ± 10.8
L0	339 ± 11.2	342 ± 9.1	341 ± 10.3	333 ± 5.7
L7	356 ± 11.7	362 ± 7.2	361 ± 9.7	358 ± 4.6
L14	353 ± 10.1	369 ± 6.5	374 ± 11.6	370 ± 5.3
PW1	327 ± 7.9	325 ± 7.0	324 ± 5.6	326 ± 9.1
PW2	334 ± 8.3	331 ± 6.6	336 ± 10.3	323 ± 8.3
PW3	334 ± 8.9	332 ± 8.4	336 ± 11.1	325 ± 8.9
PW4	335 ± 10.0	338 ± 7.8	333 ± 11.0	323 ± 8.7

[a]Mean ± SEM; N = 12.

PD: Predosing
PM: Premating
G: Gestation
L: Lactation
PW: Postweaning

Table 6. Absolute and Relative Organ Weights[a] of Male Rats Treated with MACS for 28 Days

	CONTROL	LOW	MEDIUM	HIGH
Body Wt	490 ± 15.8	507 ± 15.5	481 ± 15.2	465 ± 10.5
Brain	2.11 ± 0.03	2.15 ± 0.02	2.12 ± 0.04	2.01 ± 0.03
Ratio[b]	0.43 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.43 ± 0.01
Liver	17.90 ± 0.78	18.55 ± 1.26	17.49 ± 0.75	16.76 ± 0.92
Ratio	3.65 ± 0.08	3.64 ± 0.14	3.63 ± 0.10	3.60 ± 0.13
Kidneys	3.48 ± 0.10	3.56 ± 0.16	3.54 ± 0.12	3.52 ± 0.13
Ratio	0.71 ± 0.02	0.70 ± 0.02	0.74 ± 0.02	0.76 ± 0.02
Spleen	0.76 ± 0.03	0.81 ± 0.02	0.81 ± 0.03	0.75 ± 0.03
Ratio	0.16 ± <0.1	0.16 ±<0.1	0.17 ±<0.1	0.16 ± 0.01
Thymus	0.40 ± 0.04	0.44 ± 0.03	0.37 ± 0.03	0.41 ± 0.03
Ratio	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Testes	3.35 ± 0.10	3.40 ± 0.22	3.53 ± 0.16	3.30 ± 0.09
Ratio	0.69 ± 0.03	0.67 ± 0.04	0.73 ± 0.02	0.71 ± 0.02
Epididymis	1.33 ± 0.03	1.27 ± 0.03	1.30 ± 0.04	1.29 ± 0.03
Ratio	0.27 ±<0.1	0.25 ± 0.01	0.27 ± 0.01	0.28 ± 0.01
Rt. Cauda Epididymis	0.29 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.27 ± 0.01
Ratio	0.06 ±<0.1	0.05 ±<0.1	0.06 ±<0.1	0.06 ±<0.1

[a]Mean ± SEM, N = 6.
[b]Organ weight/body weight x 100.

Table 7. Absolute and Relative Organ Weights[a] of Male Rats Treated with MACS for 90 Days

	CONTROL	LOW	MEDIUM	HIGH
Body Wt	591 ± 18.6	583 ± 44.2	617 ± 31.2	609 ± 26.0
Brain	2.15 ± 0.05	2.09 ± 0.03	2.17 ± 0.05	2.15 ± 0.04
Ratio[b]	0.37 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.36 ± 0.01
Liver	19.80 ± 0.88	20.04 ± 0.88	20.80 ± 1.57	22.45 ± 1.34
Ratio	3.35 ± 0.13	3.43 ± 0.08	3.35 ± 0.09	3.68 ± 0.12
Kidneys	3.78 ± 0.17	4.08 ± 0.14	3.92 ± 0.23	4.04 ± 0.18
Ratio	0.64 ± 0.02	0.70 ± 0.02	0.64 ± 0.02	0.66 ± 0.01
Spleen	0.84 ± 0.03	0.87 ± 0.03	0.85 ± 0.03	0.97 ± 0.09
Ratio	0.14 ± 0.01	0.15 ±<0.1	0.14 ± 0.01	0.16 ± 0.01
Thymus	0.34 ± 0.01	0.44 ± 0.05	0.46 ± 0.05	0.51 ± 0.06
Ratio	0.06 ± <0.1	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Testes	3.49 ± 0.17	3.40 ± 0.25	3.55 ± 0.07	3.58 ± 0.12
Ratio	0.59 ± 0.03	0.59 ± 0.05	0.58 ± 0.03	0.59 ± 0.03
Epididymis	1.46 ± 0.07	1.51 ± 0.06	1.46 ± 0.04	1.48 ± 0.06
Ratio	0.25 ± 0.01	0.26 ± 0.01	0.24 ± 0.01	0.24 ± 0.01
Rt. Cauda Epididymis	0.33 ± 0.03	0.32 ± 0.01	0.31 ± 0.01	0.32 ± 0.01
Ratio	0.06 ± 0.01	0.06 ±<0.1	0.05 ±<0.1	0.05 ±<0.1

[a]Mean ± SEM, N = 6.
[b]Organ weight/body weight x 100.

Table 8. Absolute and Relative Organ Weights[a] of Female Rats Treated with MACS for 90 Days

	CONTROL	LOW	MEDIUM	HIGH
Body Wt	340 ± 10.8	340 ± 7.6	338 ± 12.3	325 ± 8.1
Brain	1.95 ± 0.04	1.95 ± 0.03	1.94 ± 0.02	1.96 ± 0.03
Ratio[b]	0.58 ± 0.01	0.58 ± 0.01	0.58 ± 0.02	0.61 ± 0.02
Liver	11.84 ± 0.48	12.06 ± 0.33	12.50 ± 0.67	12.04 ± 0.35
Ratio	3.48 ± 0.08	3.55 ± 0.08	3.67 ± 0.07	3.70 ± 0.04
Kidneys	2.27 ± 0.06	2.27 ± 0.07	2.34 ± 0.07	2.23 ± 0.05
Ratio	0.67 ± 0.02	0.67 ± 0.02	0.70 ± 0.02	0.69 ± 0.01
Spleen	0.64 ± 0.04	0.63 ± 0.03	0.64 ± 0.04	0.61 ± 0.02
Ratio	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.19 ±<0.1
Thymus	0.41 ± 0.04	0.38 ± 0.02	0.36 ± 0.03	0.42 ± 0.04
Ratio	0.12 ± 0.01	0.11 ±<0.1	0.11 ± 0.01	0.13 ± 0.01

[a]Mean ± SEM, N = 6.
[b]Organ weight/body weight x 100.

Table 9 Sperm Evaluation from Rats Administered MACS in Diet

Parameter	Control (N=6)	Low (N=6)	Medium (N=6)	High (N=6)
28 days of treatment				
Mean (±SEM) number motile cells analyzed	419.7 ± 38.6	315.5 ± 60.6	362.7 ± 54.3	399.5 ± 53.2
Mean Concentration Motile (million/ml)	1.43	1.04	1.20	1.35
Mean (±SEM) number of cells traveling in a circular pattern	45.8 ± 14.3	58.0 ± 18.3	66.5 ± 15.4	59.5 ± 10.7
Percent cells traveling in a circular pattern	11.6	16.4	17.3	17.2
Percent in circular pattern compared to total motile cells	7.1	9.0	10.5	9.6
90 days of treatment				
Mean (±SEM) Number motile cells analyzed	337.0 ± 60.9	333.7 ± 28.0	333.0 ± 44.3	388.4 ± 36.8
Mean Concentration motile (million/ml)	1.15	1.13	1.12	1.31
Mean (±SEM) number of cells traveling in a circular pattern	64.0 ± 14.0	68.2 ± 8.3	57.2 ± 10.7	88.4 ± 9.7
Percent cells traveling in a circular pattern	19.4	20.3	16.4	22.8
Percent in a circular pattern compared to total motile cells	12.6	11.5	9.7	15.6

Table 10. Blood Hematology Values[a] of Male Rats Following 28 Days of Treatment with MACS

	CONTROL	LOW	MEDIUM	HIGH
WBC (10^3)	12.3 ± 1.2	12.9 ± 1.3	15.3 ± 2.1	12.1 ± 0.7
RBC (10^6)	8.6 ± 0.1	8.6 ± 0.1	8.4 ± 0.2	8.7 ± 0.1
HGB (g/dL)	15.4 ± 0.1	15.0 ± 0.5	15.1 ± 0.3	15.3 ± 0.2
HCT (%)	49.2 ± 0.5	48.2 ± 1.6	48.5 ± 1.3	48.6 ± 0.6
MCV (fL)	57.6 ± 0.7	56.3 ± 2.0	57.5 ± 0.6	55.9 ± 0.5
MCH (pg)	18.0 ± 0.2	17.5 ± 0.7	17.9 ± 0.1	17.6 ± 0.1
MCHC (g/dL)	31.3 ± 0.1	31.1 ± 0.3	31.2 ± 0.2	31.5 ± 0.1
MetHb (%)	1.2 ±<0.1	1.1 ±<0.1	1.3 ±<0.1[c]	1.5 ±<0.1[b]
Platelets (10^3)	837.0 ± 18.8	868.0 ± 22.4	942.0 ± 76.3	946.7 ± 26.0
Neutrophils (%)	17.1 ± 2.6	13.4 ± 1.4	19.3 ± 5.7	13.3 ± 1.6
Lymphocytes (%)	75.6 ± 2.6	79.4 ± 1.8	73.3 ± 6.2	81.1 ± 1.8
Monocytes (%)	3.5 ± 0.6	3.0 ± 0.3	3.5 ± 0.3	2.0 ± 0.1
Eosinophils (%)	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Basophils (%)	0.7 ± <0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1

[a]Mean ± SEM, N=6.
[b]Different from control, $p<0.01$.
[c]Different form control, $p<0.05$.

Table 11. Blood Hematology Values[a] of Male Rats Following 90 Days of Treatment with MACS

	CONTROL	LOW	MEDIUM	HIGH
WBC (10^3)	11.7 ± 1.0	11.8 ± 0.5	12.6 ± 0.9	10.3 ± 0.9
RBC (10^6)	8.9 ± 0.2	9.0 ± 0.2	8.7 ± 0.1	8.5 ± 0.2
HGB (g/dL)	15.3 ± 0.3	15.3 ± 0.3	14.6 ± 0.2	14.1 ± 0.2
HCT (%)	50.1 ± 1.0	49.7 ± 1.0	47.7 ± 0.7	46.0 ± 0.6
MCV (fL)	56.2 ± 0.9	55.2 ± 0.4	60.0 ± 5.4	54.5 ± 1.2
MCH (pg)	17.2 ± 0.3	17.0 ± 0.2	16.9 ± 0.3	16.6 ± 0.5
MCHC (g/dL)	30.6 ± 0.2	30.7 ± 0.1	30.7 ± 0.2	30.5 ± 0.2
MetHb(%)	1.3 ±<0.1	1.4 ±<0.1	1.5 ±<0.1	1.6 ±<0.1[b]
Platelets (10^3)	901.8 ± 62.5	925.5 ± 48.5	887.3 ± 68.4	951.5 ± 45.5
Neutrophils (%)	17.8 ± 3.7	10.6 ± 0.9	13.3 ± 2.6	12.2 ± 2.2
Lymphocytes (%)	75.0 ± 3.8	82.5 ± 0.9	78.4 ± 3.0	80.8 ± 2.5
Monocytes (%)	3.1 ± 0.4	3.2 ± 0.4	3.6 ± 0.5	2.7 ± 0.3
Eosinophils (%)	1.0 ± 0.1	0.9 ± 0.1	1.2 ± 0.2	1.2 ± 0.3
Basophils (%)	0.6 ± <0.1	0.6 ± 0.1	0.6 ± <0.1	0.6 ± <0.1

[a]Mean ± SEM, N=6.
[b]Different from control, $p<0.01$.

Table 12. Blood Hematology Values[a] of Female Rats Following 90 Days of Treatment with MACS

	CONTROL	LOW	MEDIUM	HIGH
WBC (10^3)	9.1 ± 0.7	8.2 ± 0.5	8.7 ± 0.6	9.2 ± 0.5
RBC (10^6)	8.0 ± 0.1	7.9 ± 0.1	8.0 ± 0.1	7.8 ± 0.1
HGB (g/dL)	14.3 ± 0.2	14.2 ± 0.1	14.1 ± 0.1	13.8 ± 0.2
HCT (%)	45.0 ± 0.8	44.7 ± 0.5	44.4 ± 0.3	43.5 ± 0.6
MCV (fL)	52.6 ± 1.7	55.3 ± 1.0	52.0 ± 1.6	54.8 ± 1.1
MCH (pg)	18.9 ± 0.2	17.9 ± 0.2	17.8 ± 0.2	17.7 ± 0.1
MCHC (g/dL)	31.9 ± 0.2	31.8 ± 0.2	31.8 ± 0.2	31.6 ± 0.1
MetHb (%)	1.5 ±<0.1	1.5 ±<0.1	1.8 ±<0.1[b]	1.9 ±<0.1[b]
Platelets (10^3)	894.3 ± 24.4	851.0 ± 29.3	887.3 ± 32.8	842.7 ± 32.6
Neutrophils (%)	7.7 ± 0.7	11.3 ± 1.4	11.2 ± 1.3	10.2 ± 1.4
Lymphocytes (%)	84.8 ± 0.8	82.2 ± 1.7	80.9 ± 1.8	82.1 ± 2.1
Monocytes (%)	3.7 ± 0.4	3.4 ± 0.3	3.2 ± 0.3	3.6 ± 0.5
Eosinophils (%)	1.0 ± 0.1	1.1 ± 0.1	2.0 ± 0.9	1.1 ± 0.1
Basophils (%)	0.6 ± <0.1	0.6 ± <0.1	0.6 ± <0.1	0.5 ± <0.1

[a]Mean ± SEM, N=12.
[b]Different from control, $p<0.01$.

Table 13. Mean Values[a] of Serum Chemistry Parameters for Male Rats Following 28 Days of Treatment with MACS

	CONTROL	LOW	MEDIUM	HIGH
BUN (mg/kg)	15.2 ± 0.7	13.8 ± 0.9	15.7 ± 0.8	14.0 ± 0.7
Creatinine (mg/dL)	0.4 ± <0.1	0.4 ± <0.1	0.4 ± <0.1	0.4 ± <0.1
Chloride (mmol/L)	100.2 ± 0.9	100.8 ± 0.5	99.3 ± 0.9	99.2 ± 0.7
Calcium (mg/dL)	11.2 ± 0.1	11.2 ± 0.2	11.1 ± 0.2	11.1 ± 0.2
Phosphorus (mg/dL)	11.5 ± 0.8	11.6 ± 0.2	12.3 ± 0.6	12.5 ± 0.9
Total Protein (g/dL)	6.4 ± 0.1	6.5 ± 0.2	6.5 ± 0.2	6.3 ± 0.1
AST (IU/L)	87.8 ± 4.5	91.7 ± 6.1	93.3 ± 8.6	85.0 ± 8.4
ALT (IU/L)	52.7 ± 3.8	56.0 ± 3.9	54.3 ± 4.9	47.3 ± 4.0[b]
Alkaline phosphatase (IU/L)	203.8 ± 14.1	187.8 ± 19.1	214.2 ± 22.9	206.0 ± 17.9
Glucose (mg/dL)	178.0 ± 7.3	169.2 ± 11.2	155.3 ± 11.9	152.0 ± 6.4
Sodium (mmol/L)	148.5 ± 1.1	148.3 ± 1.1	147.0 ± 0.5	147.5 ± 0.4
Triglycerides (mg/dL)	107.7 ± 13.2	124.8 ± 32.1	100.8 ± 10.6	105.7 ± 19.8
Magnesium (mg/dL)	3.3 ± 0.2	3.3 ± 0.2	3.3 ± 0.1	3.5 ± 0.2
Potassium (mmol/L)	7.0 ± 0.5	7.7 ± 0.5	7.9 ± 0.3	7.4 ± 0.6
Cholesterol (mg/dL)	60.2 ± 2.5	63.0 ± 2.9	58.8 ± 5.6	54.5 ± 1.1
Total Bilirubin (mg/dL)	0.4 ± <0.1	0.3 ± <0.1	0.4 ± <0.1	0.5 ± 0.1
CO_2 (IU/L)	33.5 ± 1.4	33.7 ± 1.3	32.2 ± 1.6	33.5 ± 1.8
Uric Acid (mg/dL)	2.0 ± 0.3	2.1 ± 0.2	2.0 ± 0.2	2.3 ± 0.3
Albumin (g/dL)	3.5 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1
Globulin (g/dL)	2.9 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	2.8 ± 0.1
Creatine Kinase (IU/L)	78.5 ± 4.6	107.5 ± 14.4	98.7 ± 14.3	101.2 ± 11.4
GGT (IU/L)	9.0 ± <0.1	9.0 ± <0.1	9.0 ± <0.1	9.0 ± <0.1

[a]Mean ± SEM, N=6.
[b]Different from control, $p<0.05$.

Table 14. Mean Values[a] of Serum Chemistry Parameters for Male Rats Following 90 Days of Treatment with MACS

	CONTROL	LOW	MEDIUM	HIGH
BUN (mg/kg)	15.0 ± 0.8	14.8 ± 0.7	13.7 ± 0.6	15.2 ± 0.6
Creatinine (mg/dL)	0.4 ± <0.1	0.4 ± <0.1	0.4 ± <0.1	0.4 ± <0.1
Chloride (mmol/L)	100.3 ± 0.3	99.7 ± 0.6	100.0 ± 0.7	99.8 ± 0.7
Calcium (mg/dL)	11.0 ± 0.1	11.4 ± 0.1	11.3 ± 0.1	11.2 ± 0.1
Phosphorus (mg/dL)	9.2 ± 0.3	9.5 ± 0.3	9.2 ± 0.2	9.2 ± 0.3
Total Protein (g/dL)	6.8 ± 0.1	6.7 ± 0.2	6.8 ± 0.1	6.8 ± 0.2
AST (IU/L)	104.7 ± 5.9	87.2 ± 6.9	88.2 ± 9.9	69.8 ± 2.3
ALT (IU/L)	72.0 ± 7.7	60.2 ± 5.8	55.8 ± 10.2	43.2 ± 3.4[b]
Alkaline phosphatase (IU/L)	194.5 ± 16.5	213.2 ± 16.7	162.0 ± 11.7	182.2 ± 22.8
Glucose (mg/dL)	180.0 ± 15.6	191.5 ± 3.2	181.7 ± 5.0	167.2 ± 11.4
Sodium (mmol/L)	148.5 ± 0.7	147.3 ± 0.3	147.3 ± 0.7	148.2 ± 0.4
Triglycerides (mg/dL)	184.2 ± 37.5	181.2 ± 31.8	172.8 ± 30.6	156.5 ± 21.3
Magnesium (mg/dL)	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1
Potassium (mmol/L)	6.7 ± 0.3	7.2 ± 0.5	6.4 ± 0.4	6.4 ± 0.3
Cholesterol (mg/dL)	62.7 ± 3.3	58.0 ± 2.7	66.7 ± 4.9	63.3 ± 3.7
Total Bilirubin (mg/dL)	0.4 ± <0.1	0.4 ± 0.1	0.4 ± <0.1	0.5 ± 0.1
CO_2 (IU/L)	36.8 ± 0.7	36.5 ± 1.0	36.2 ± 0.9	33.5 ± 1.2
Uric Acid (mg/dL)	1.4 ± 0.2	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.2
Albumin (g/dL)	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.6 ± 0.1
Globulin (g/dL)	3.3 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	3.2 ± 0.1
Creatine Kinase (IU/L)	87.0 ± 6.9	60.2 ± 12.5	45.2 ± 6.3	43.0 ± 5.1
GGT (IU/L)	8.0 ± <0.1	8.0 ± <0.1	8.0 ± <0.1	8.0 ± <0.1

[a]Mean ± SEM, N=6.
[b]Different from control, $p<0.05$.

Table 15. Mean Values[a] of Serum Chemistry Parameters for Female Rats Following 90 Days of Treatment with MACS

	CONTROL	LOW	MEDIUM	HIGH
BUN (mg/kg)	15.1 ± 0.7	14.6 ± 0.7	15.8 ± 0.6	13.9 ± 0.5
Creatinine (mg/dL)	0.4 ± <0.1	0.5 ± <0.1	0.5 ± <0.1	0.4 ± <0.1
Chloride (mmol/L)	100.5 ± 0.5	100.2 ± 0.4	100.3 ± 0.5	100.0 ± 0.5
Calcium (mg/dL)	11.2 ± 0.1	11.4 ± 0.1	11.4 ± 0.1	11.3 ± 0.1
Phosphorus (mg/dL)	6.6 ± 0.3	7.2 ± 0.3	6.3 ± 0.2	7.1 ± 0.3
Total Protein (g/dL)	7.4 ± 0.1	7.5 ± 0.1	7.8 ± 0.2	7.8 ± 0.1
AST (IU/L)	75.1 ± 2.6	87.7 ± 6.0	79.3 ± 4.0	81.4 ± 7.0
ALT (IU/L)	46.7 ± 2.1	51.4 ± 3.6	55.7 ± 7.8	54.0 ± 8.9
Alkaline phosphatase (IU/L)	141.7 ± 16.3	139.7 ± 15.2	135.3 ± 9.9	105.0 ± 6.4
Glucose (mg/dL)	174.7 ± 6.4	169.6 ± 4.5	171.5 ± 5.4	159.8 ± 3.7
Sodium (mmol/L)	149.0 ± 0.5	48.4 ± 0.3	148.9 ± 0.4	148.9 ± 0.5
Triglycerides (mg/dL)	154.7 ± 19.1	122.8 ± 12.6	152.1 ± 35.1	111.7 ± 10.9
Magnesium (mg/dL)	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.7 ± 0.1
Potassium (mmol/L)	5.7 ± 0.2	6.0 ± 0.2	5.7 ± 0.2	5.8 ± 0.1
Cholesterol (mg/dL)	72.0 ± 2.8	73.2 ± 4.0	79.3 ± 4.9	76.2 ± 4.4
Total Bilirubin (mg/dL)	0.4 ± <0.1	0.4 ± <0.1	0.4 ± <0.1	0.4 ± <0.1
CO_2 (IU/L)	35.6 ± 0.8	36.5 ± 0.8	36.1 ± 0.8	36.5 ± 0.7
Uric Acid (mg/dL)	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1
Albumin (g/dL)	3.9 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
Globulin (g/dL)	3.4 ± 0.1	3.4 ± 0.1	3.6 ± 0.1	3.5 ± <0.1
Creatine Kinase (IU/L)	34.5 ± 3.3	35.8 ± 8.9	30.8 ± 5.7	28.7 ± 4.4
GGT (IU/L)	8.0 ± <0.1	8.0 ± <0.1	8.0 ± 0.3	8.0 ± <0.1

[a]Mean ± SEM, N=6.

Table 16. Litter Data for Rats Treated with MACS

PARAMETER	DOSE LEVEL CONTROL	LOW	MEDIUM	HIGH
No. of mated pairs	12	12	12	12
No. dams w/ pups born	11	11	12	11
No. dams w/ pups born live	11	11	12	11
Fertility index (%)	91.7	91.7	100.0	91.7
Gestation index[a] (%)	91.7	91.7	100.0	91.7
Live Birth Index[b] (%)	97.5	98.8	93.1	97.7
4-Day Survival Index	99.4	98.1	97.8	98.8
7-Day Survival Index	100.0	100.0	100.0	100.0
14-Day Survival Index	100.0	100.0	100.0	100.0
21-Day Survival Index	100.0	100.0	100.0	100.0
Lactation Index (%)[c]	100.0	100.0	100.0	100.0
Mean no. pups / litter	14.4	14.8	15.8	15.5
Mean no. gestation days	22	22	23	22
Sex ratio (M/F)	1.00	1.16	1.20	1.05

[a] $\frac{\text{Number of females with live litters}}{\text{Number of females pregnant}}$

[b] $\frac{\text{Number of live pups at birth}}{\text{Total number of pups born}}$

[c] $\frac{\text{Number of pups surviving 21 days}}{\text{Number of pups surviving 4 days}}$

Table 17. Mean Body Weights[a] of Male and Female Rat Pups

DAY	CONTROL	LOW	MEDIUM	HIGH
		MALE		
Day 1	7.32 ± 0.10	7.53 ± 0.08	7.07 ± 0.09	7.26 ± 0.09
N	78	88	99	87
Day 4	11.05 ± 0.21	11.55 ± 0.22	10.41 ± 0.22	10.99 ± 0.21
N	43	44	50	47
Day 7	17.90 ± 0.32	19.69 ± 0.29	16.78 ± 0.39	19.47 ± 1.06
N	43	44	50	47
Day 14	34.15 ± 0.61	39.36 ± 0.40	34.99 ± 0.65	36.24 ± 0.50
N	43	44	50	47
Day 21	58.65 ± 1.12	64.03 ± 0.62	57.32 ± 1.13	59.79 ± 0.82
N	43	44	50	47
		FEMALE		
Day1	7.02 ± 0.10	7.31 ± 0.10	6.61 ± 0.08	6.82 ± 0.08
N	76	71	84	78
Day 4	10.59 ± 0.23	11.33 ± 0.21	10.05 ± 0.19	10.25 ± 0.21
N	45	44	46	41
Day 7	17.14 ± 0.34	19.11 ± 0.28	16.32 ± 0.34	16.76 ± 0.36
N	45	44	46	41
Day 14	33.30 ± 0.59	38.33 ± 0.45	34.1 ± 0.58	34.70 ± 0.52
N	45	44	46	41
Day 21	55.51 ± 0.92	61.50 ± 0.70	55.71 ± 0.98	56.50 ± 0.87
N	45	44	46	41

[a]Mean ± SEM.

Appendix A

MACS composition as listed in the Hercules Material Safety Data Sheet dated December 15, 1992 is:

47.7% Nitroguanidine
28.0% Nitrocellulose
22.5% Nitroglycerin
1.5% Ethylcentralite
0.3% Cryolite
0.2% Graphite

An analysis of the MACS material used to prepare the rat diets indicated 46.4% nitroguanidine. The main component, nitroguanidine, was detectable by spectrometry (UV absorption at 265 nm wavelength). Because two secondary components are not readily detected by absorption, the MACS analysis was based on the nitroguanidine analysis.

External standards were prepared by adding weighed amounts of MACS to 5.0 g rodent diet and 10-mL of methanol in a 20-mL scintillation vial. The standards were mixed for 20 min on a Haak-Buchler (Haak-Buchler Instruments, Inc., Saddlebrook, NJ) vortex mixer. After centrifugation for 5 min at 2200 rpm, 1-mL samples were pipetted into 2-mL autosampler vials. Control diet samples and treated diet samples were treated similarly.

Samples were analyzed using a Hewlett-Packard High Performance Liquid Chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a 4.6-mm x 220-mm Spheri 5 RP-8S, 5μ particle size, reverse phase column (Alltech Associates, Inc., Deerfield, IL). The carrier flow was set at 0.4 mL/min of 50/50 methanol and water with an injection volume of 1.0 μL. A variable wavelength detector set at 265 nm provided maximum sensitivity for nitroguanidine. All rodent diet analysis concentrations are expressed as mg MACS/g rodent chow.